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***IN THE UNITED STATES PATENT AND TRADEMARK OFFICE***

Applicant: Kazuo YAMAMOTO, et al.  
Title: CARBOHYDRATE LIBRARY  
CONSTRUCTED BY GENE  
ALTERATION OF CARGO  
RECEPTORS  
Appl. No.: Unassigned  
Filing Date: 2/18/2005  
Examiner: Unassigned  
Art Unit: Unassigned

**PETITION TO ACCEPT COLOR DRAWINGS UNDER 37 CFR 1.84**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

In accordance with 37 CFR 1.84(a)(2), Applicants petition for the acceptance of color photographs to be entered in the above-captioned application.

In support thereof, Applicants submit:

- I) A fee in the amount of \$130.00 as is set forth under 37 CFR 1.17(h);
- II) Three sets of Figures 1-16 (17 pages), all of which are in color;
- III) An amendment to the specification indicating that the application contains color drawings.

03/01/2005 LLANDGRA 00000009 10525020

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In the Specification:

On page 1, directly beneath the title, please insert the following

--The application contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.--

**REMARKS**

Fig. 1 shows cargo receptors (ERGIC-53 and VIP36) involved in carbohydrate processing of glycoproteins and the quality control of carbohydrates (sugars) and the outline of secretory pathway.

Fig. 2 shows the outline of VIP36 library construction.

Fig. 3 shows the result of introducing random mutations into VIP36. "A" shows the nucleotide sequences of the putative carbohydrate-binding domains of VIP36 and "B" shows the amino acid sequences thereof.

Fig. 4 shows photographs of overexpression of altered VIP36 in MDCK cells.

Fig. 5 shows the binding of plant lectins to lectin-positive (red) and lectin-negative (black) MDCK cells. The upper row shows untransfected cells, the middle row shows mutated VIP36 transfected cells, and the lower row shows untransfected control MDCK cells.

Fig. 6 shows the binding of PHA-E4 and WGA to lectin-positive (red) and lectin-negative (black) MDCK cells. The uppermost row shows wild-type cells, the second row from the top shows mutated VIP36 transfected cells resulting from the 1st separation, the second row from the bottom shows mutated VIP36 transfected cells resulting from the 2nd separation, and the lowermost row shows the mutated VIP36 transfected cells resulting from the 3rd separation.

Fig. 7 shows the effect of trypsinization on the binding of lectins. PBS-EDTA treatment is indicated with red, and trypsin-EDTA treatment is indicated with blue.

Fig. 8 is an outline showing the design of random primers for introducing random mutations into the carbohydrate-binding domain of ERGIC-53.

Fig. 9A shows a photograph showing samples prepared using an anti-FLAG antibody as a primary antibody and a goat anti-mouse IgG1-FITC as a secondary antibody and then observed by a fluorescence microscope. As a negative control, wild-type MDCK cells were observed.

Fig. 9B shows a photograph showing samples prepared using an anti-FLAG antibody as a primary antibody and goat anti-mouse IgG1-FITC as a secondary antibody and then observed by a fluorescence microscope. As a positive control, VIP36-FLAG clone8 (clones whose constant expression has been confirmed) was observed.

Fig. 9C shows photographs showing samples prepared using an anti-FLAG antibody as a primary antibody and goat anti-mouse IgG1-FITC as a secondary antibody and then observed by a fluorescence microscope. ERGIC random libraries were transfected into MDCK cells, and then selection was carried out for 10 days with 1.5 mg/ml G418, and then the cells were observed.

Fig. 10 shows histograms showing the result of analyzing the cells following MACS screening using various biotinylated lectins as primary antibodies and FITC-labeled streptavidin as a secondary antibody. A black line indicates control MDCK cells, a red line indicates a (-) fraction following MACS, and a green line indicates a (+) fraction.

Fig. 11 shows a histogram showing the result of analyzing by FACS MDCK cells fractionated by MACS using PNA lectins. The binding strength of PNA (-), that of PNA (+), and that of PNA2 (+) to PNA were compared.

Fig. 12 shows photographs showing the results of western blotting carried out for PNA (-), PNA (+), and PNA2 (+) using 5 types of biotinylated lectins as primary antibodies and streptavidin alkaline phosphatase as a secondary antibody.

Fig. 13 shows the result of analyzing the carbohydrate-binding specificity of each cell fraction when FACS was carried out for a control, PNA (-), PNA (+), and PNA2 (+) using MAM or PNA as a primary antibody and streptavidin FITC as a secondary antibody.


Fig. 14 shows the outline of a technique for separating cells having specific carbohydrate moieties by flow cytometry or a magnetic cell sorting (MACS) using labeled lectins.

Fig. 15 shows the intensity of fluorescence for labeled PNA lectins in a process where cells having carbohydrate moieties specifically binding to PNA lectins were separated by flow cytometry (FACS) and then enriched.

Fig. 16 shows the expression of carbohydrate moieties to be recognized by altered VIP36 and PNA lectins in clone 12 and control CHO cells.

The results of these comparisons and measurements are best depicted in color and the photographs enhance visual contrast, which otherwise would be indistinct. Favorable action is solicited.

Respectfully submitted,

By 

Date February 18, 2005

FOLEY & LARDNER LLP  
Customer Number: 22428  
Telephone: (202) 672-5404  
Facsimile: (202) 672-5399

Stephen A. Bent  
Attorney for Applicant  
Registration No. 29,768

FIG 1

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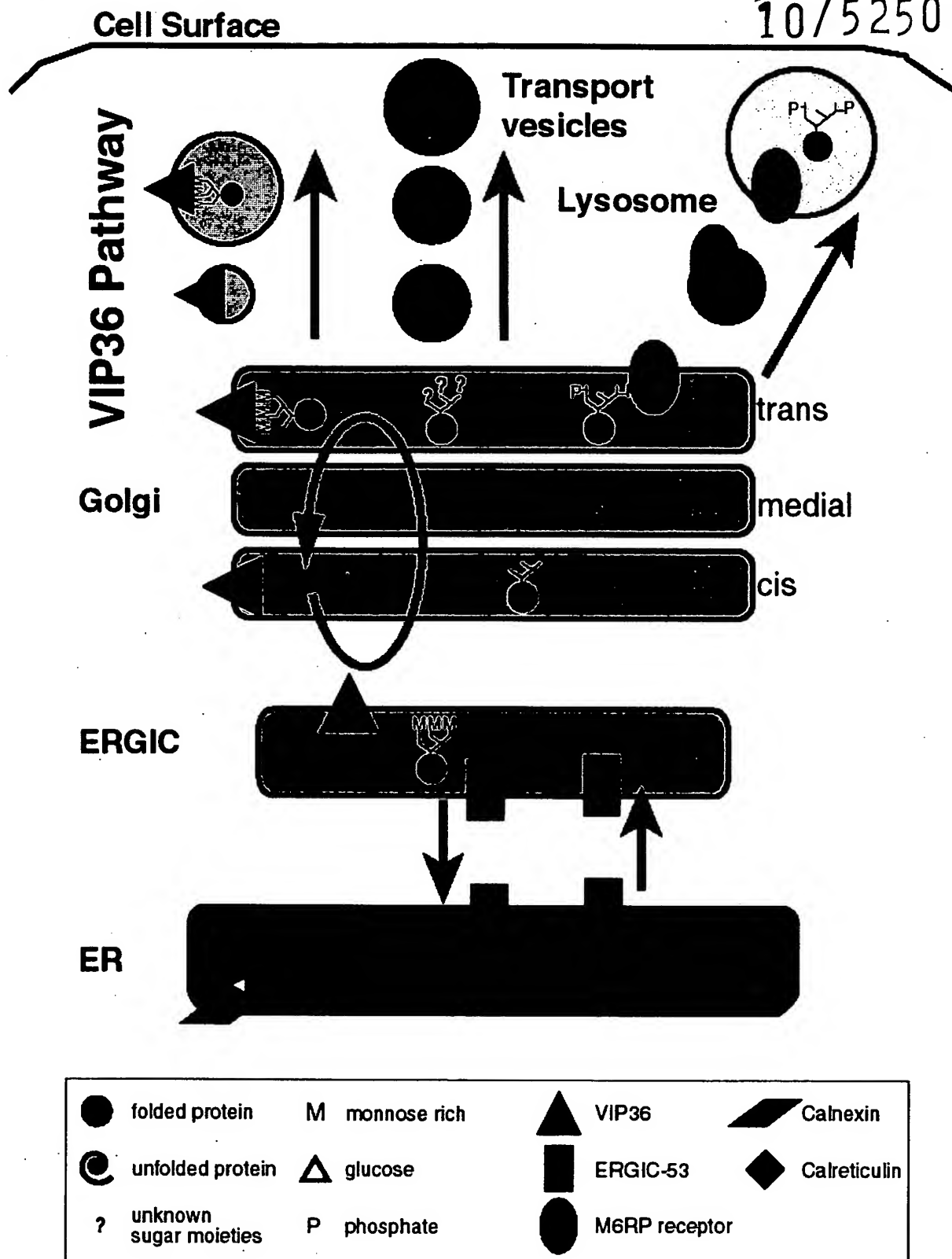
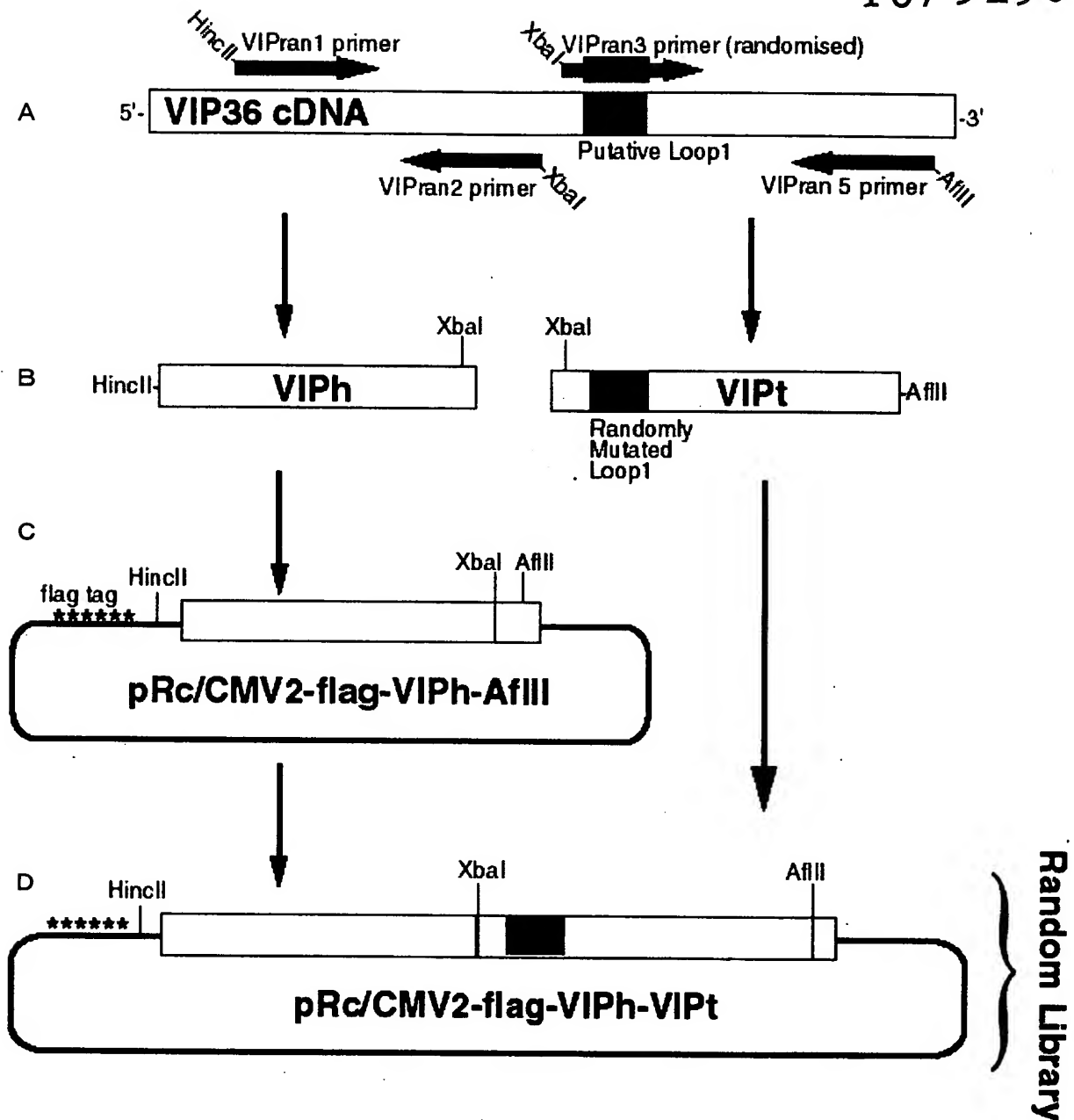


FIG 2

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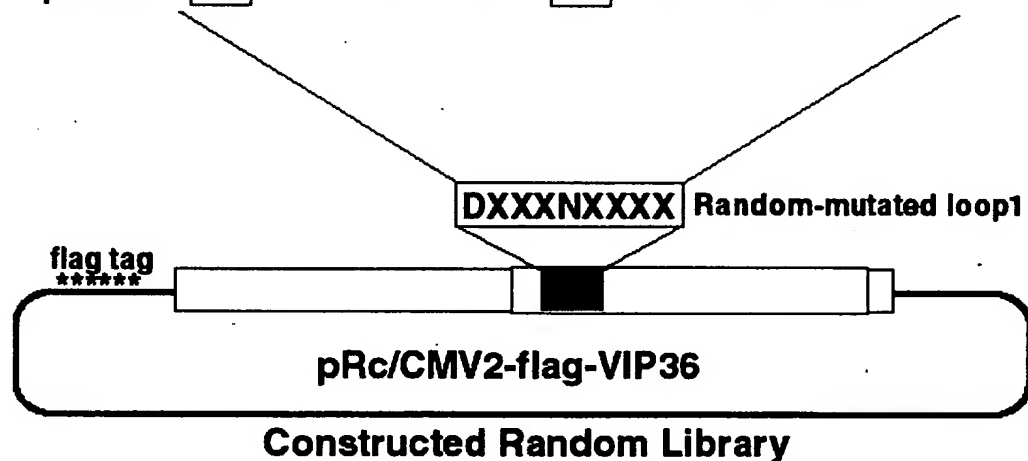
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**A**

VIP36 original	GAC	ACC	TAC	CCC	AAT	GAT	GAG	ACC	ACT
vip3f clone	GAC	AGG	CAT	GGG	AAT	TAT	AGT	GTT	TAT
vip26f clone	GAC	ATT	CGT	TTG	AAT	CTT	GAG	AGT	GGT
vip28f clone	GAC	CGT	GCG	CTT	AAT	CTT	ATG	CTT	CGT
B8-VIP10.Seq clone	GAC	GTG	TTT	GCT	AAT	CAT	TGT	CAT	TTG
B2-VIP7.Seq clone	GAC	-TG	TGT	GGT	AAT	TGT	GGG	TGT	TAG
B6-VIP9.Seq clone	GAC	CAT	GGG	TTT	AAT	GCT	AAT	CGG	ATT
A3-VIP2.Seq clone	GAC	GTG	TGT	TGG	AAT	GGG	GGG	TGT	AAT

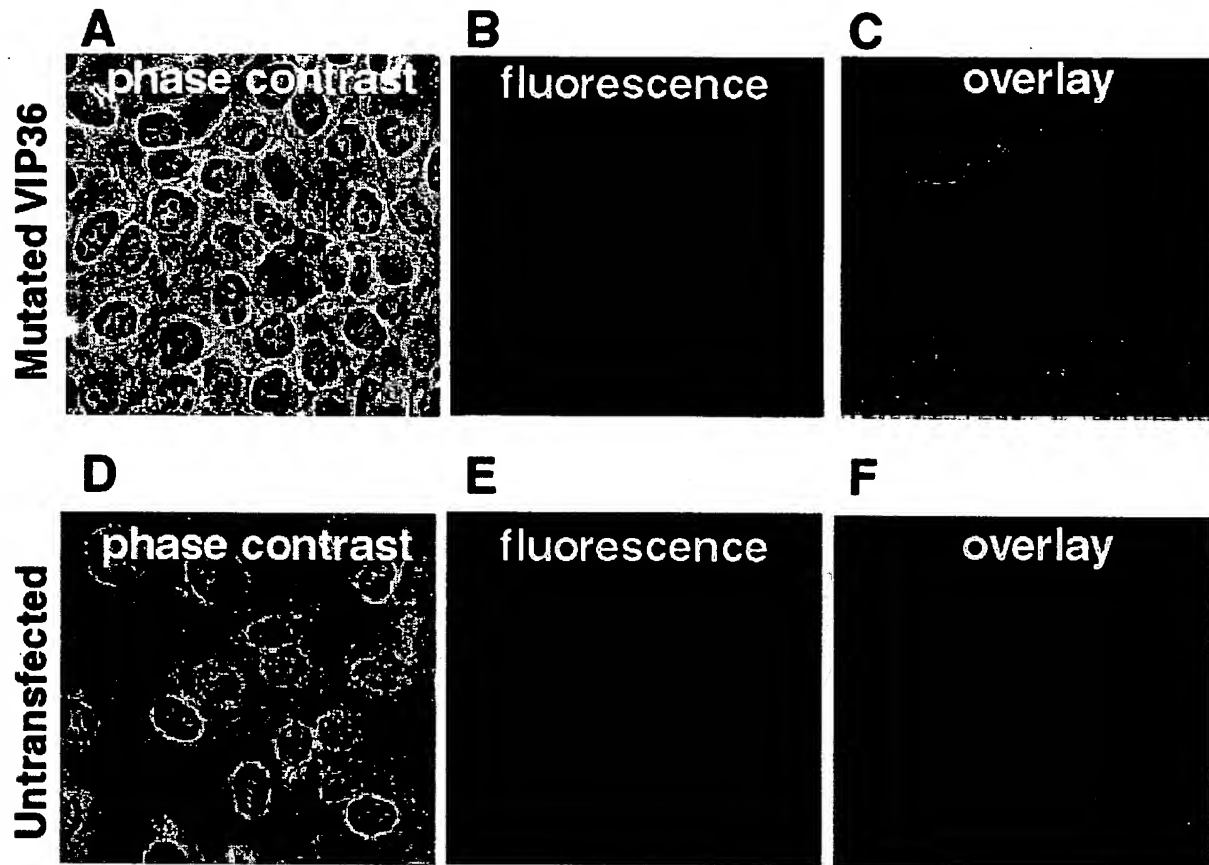
**B**

VIP36 original	D	T	Y	P	N	D	E	T	T
vip3f clone	D	R	H	G	N	Y	S	V	Y
vip26f clone	D	I	R	L	N	L	E	S	G
vip28f clone	D	R	A	L	N	L	M	L	R
B8-VIP10.Seq clone	D	T	Y	P	N	D	E	T	T
B2-VIP7.Seq clone	D	-	C	G	N	C	G	C	*
B6-VIP9.Seq clone	D	H	G	F	N	A	N	R	I
A3-VIP2.Seq clone	D	V	C	W	N	G	G	C	N



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FIG 4





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FIG 5

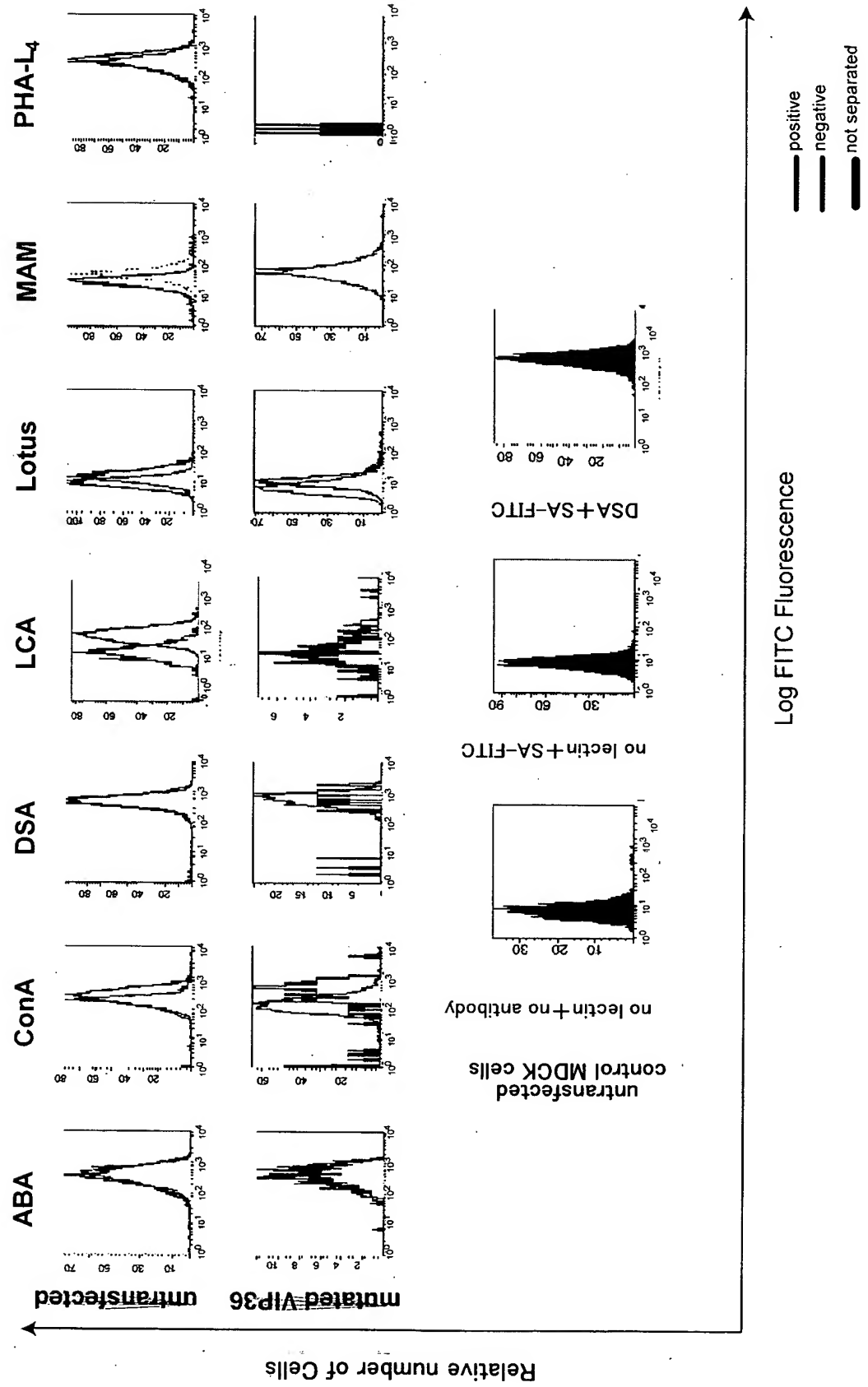


FIG 6

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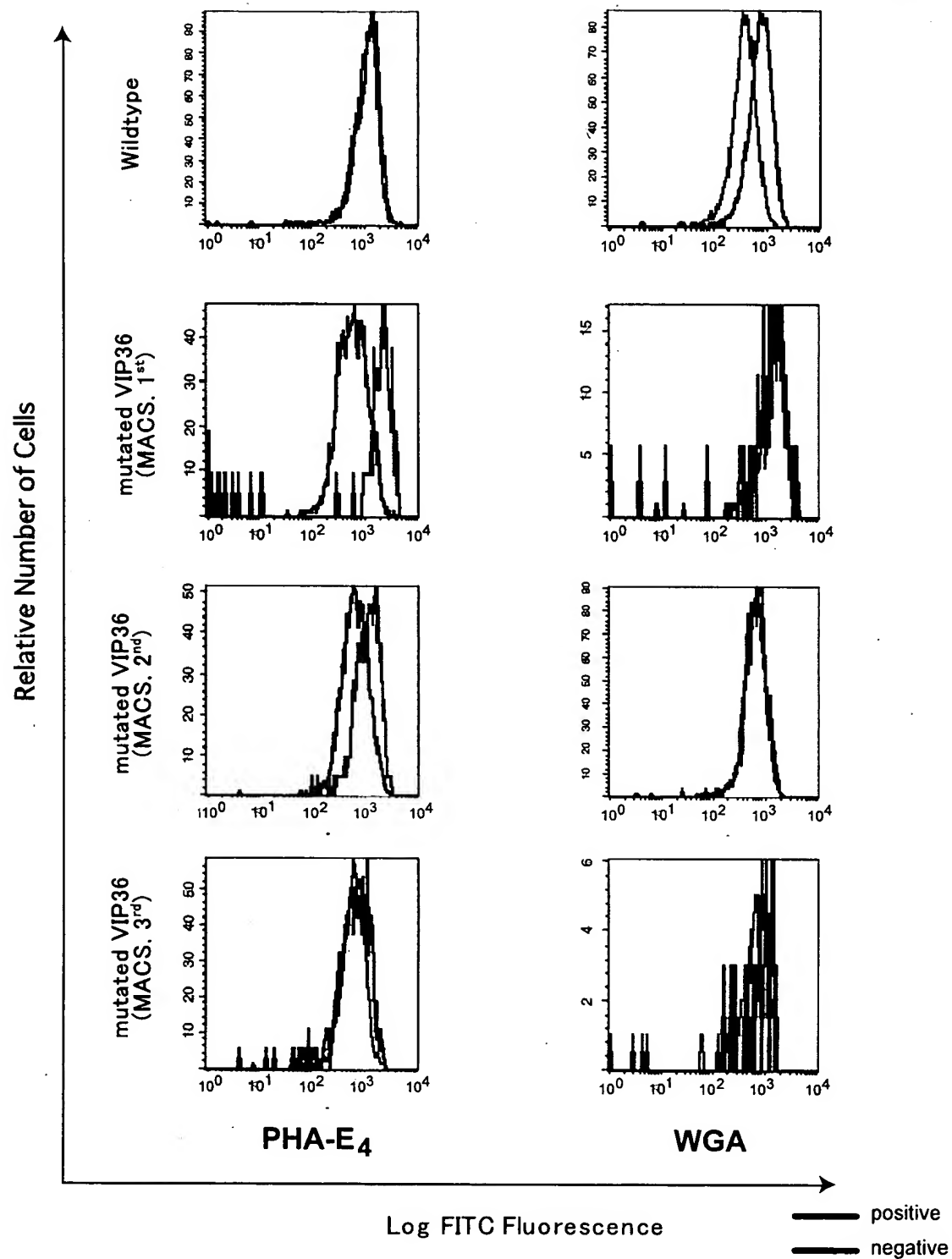
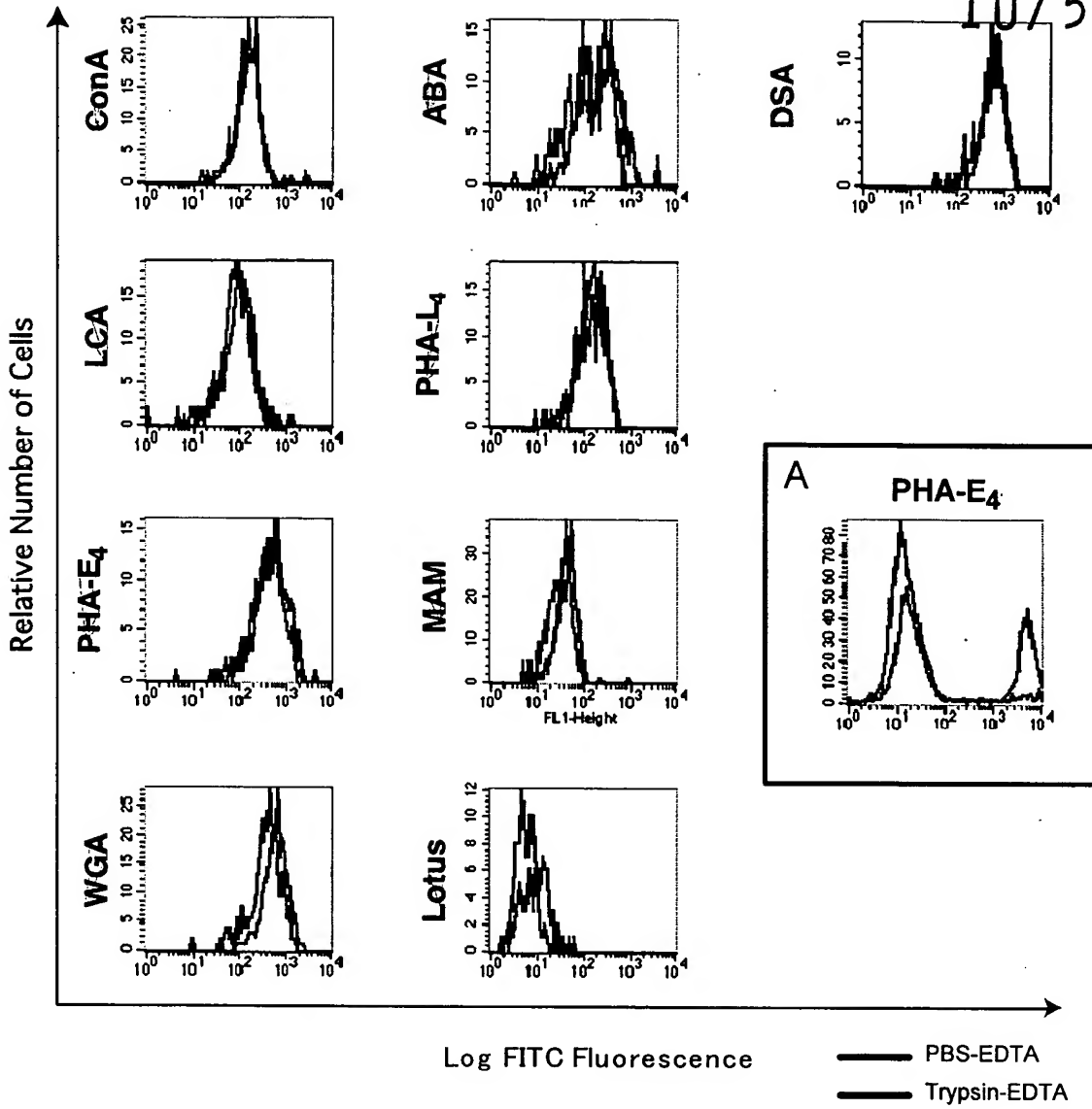
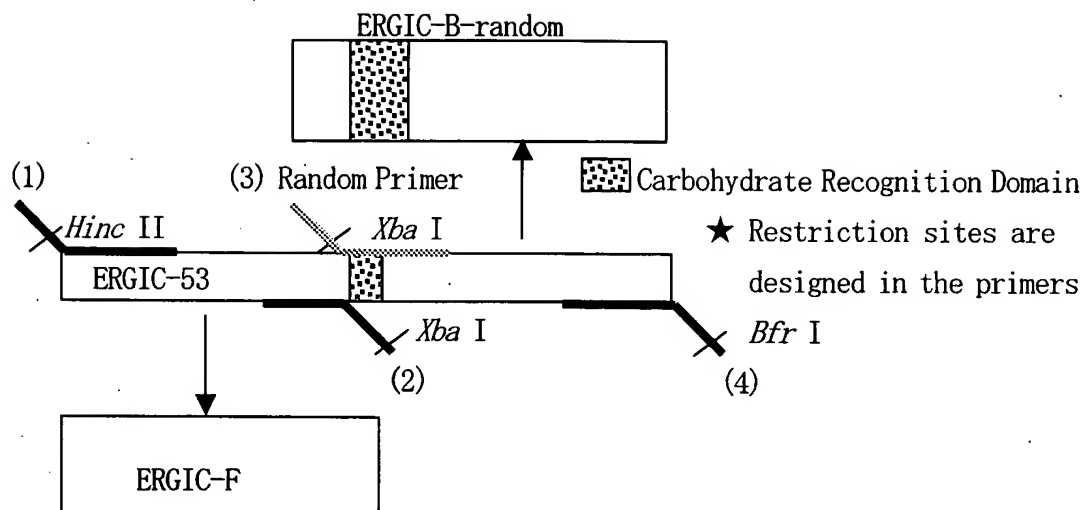


FIG 7

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FIG 9 A



FIG 9 B



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FIG 9 C

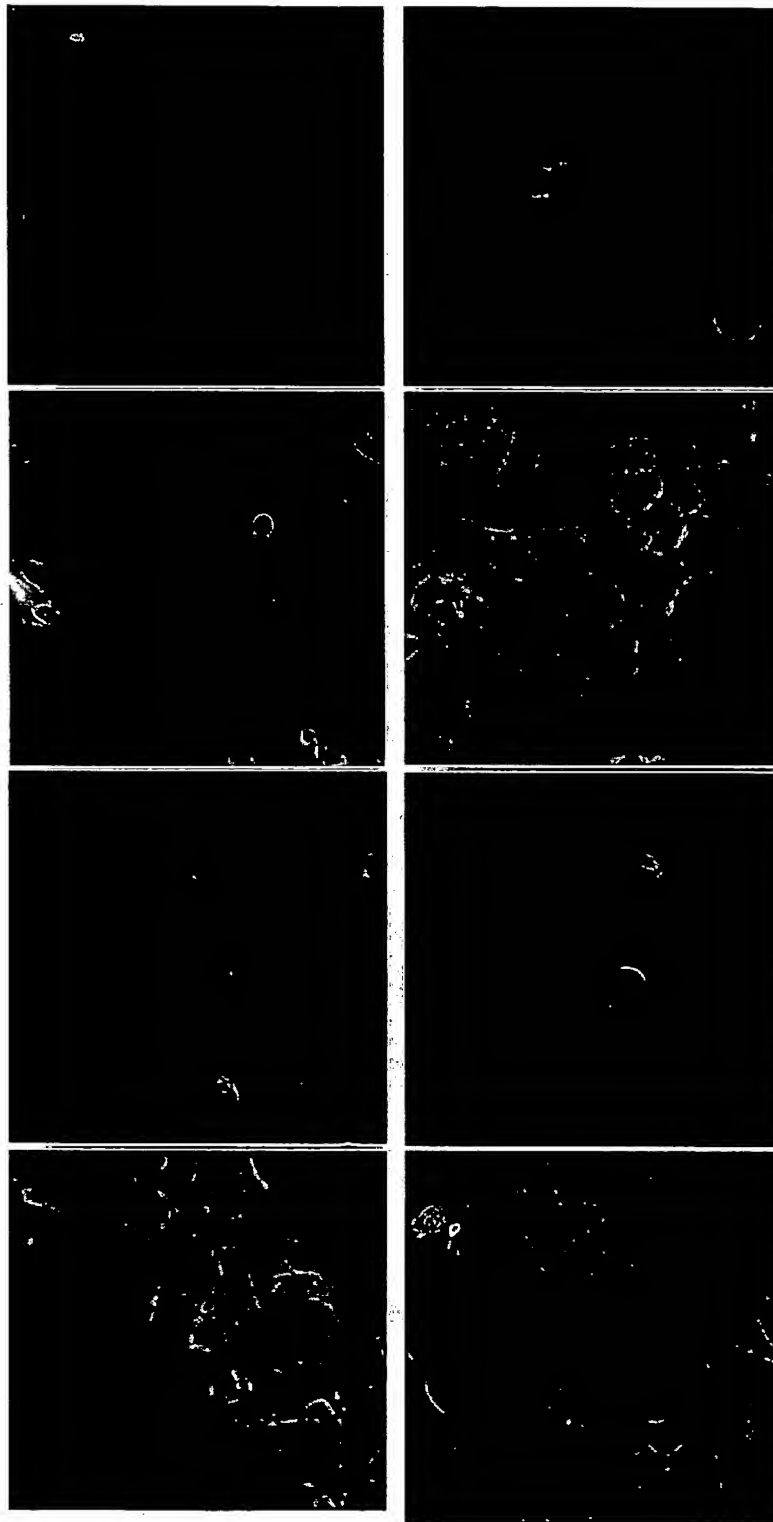
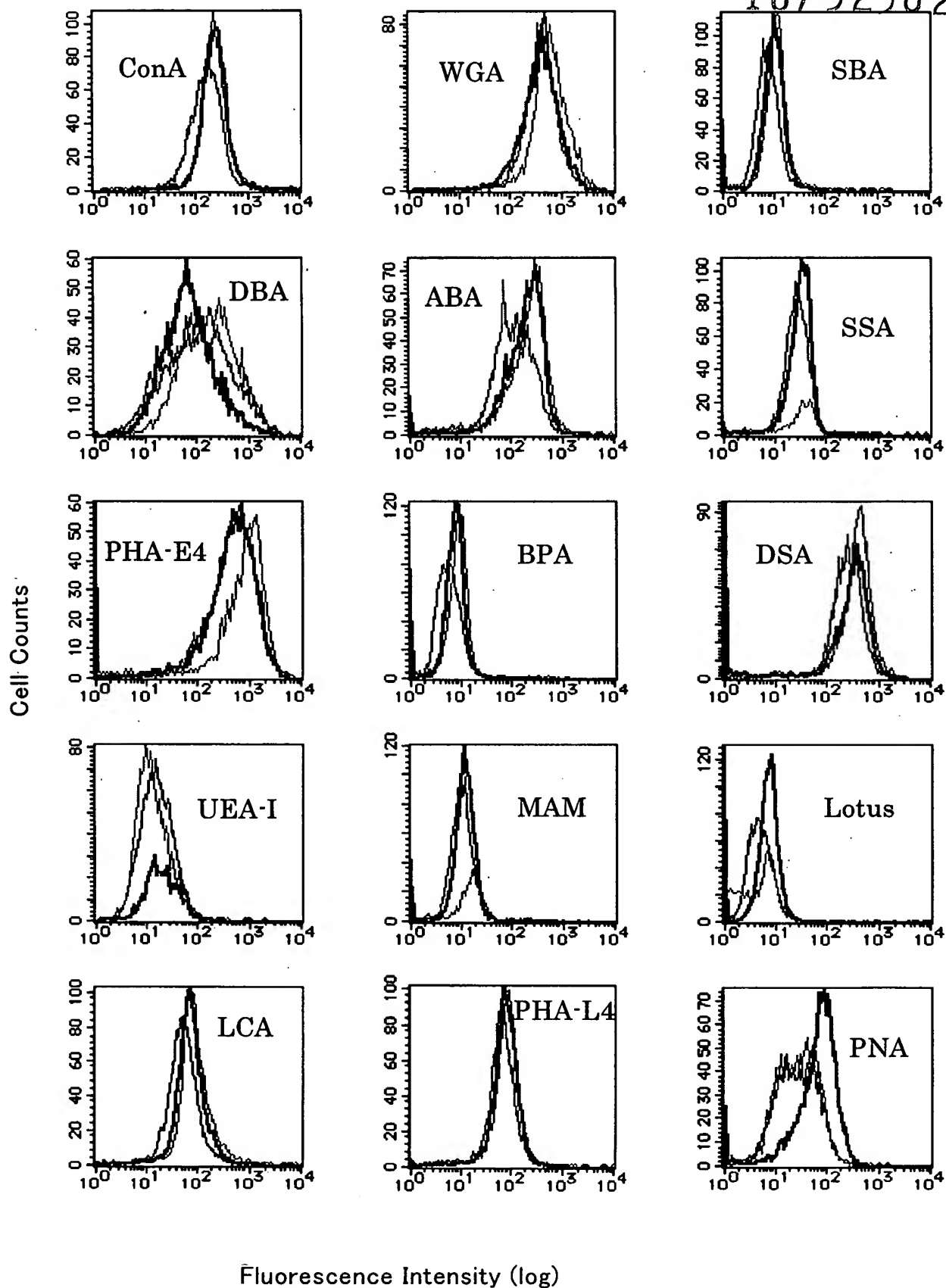


FIG 10

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FIG 11

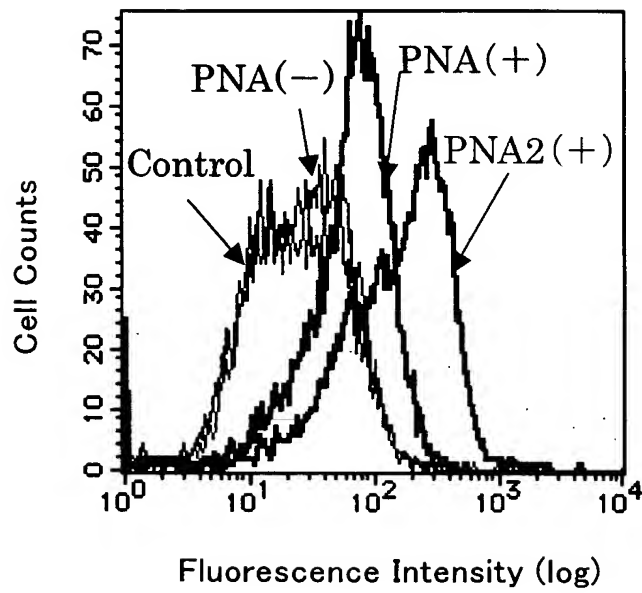




FIG 12

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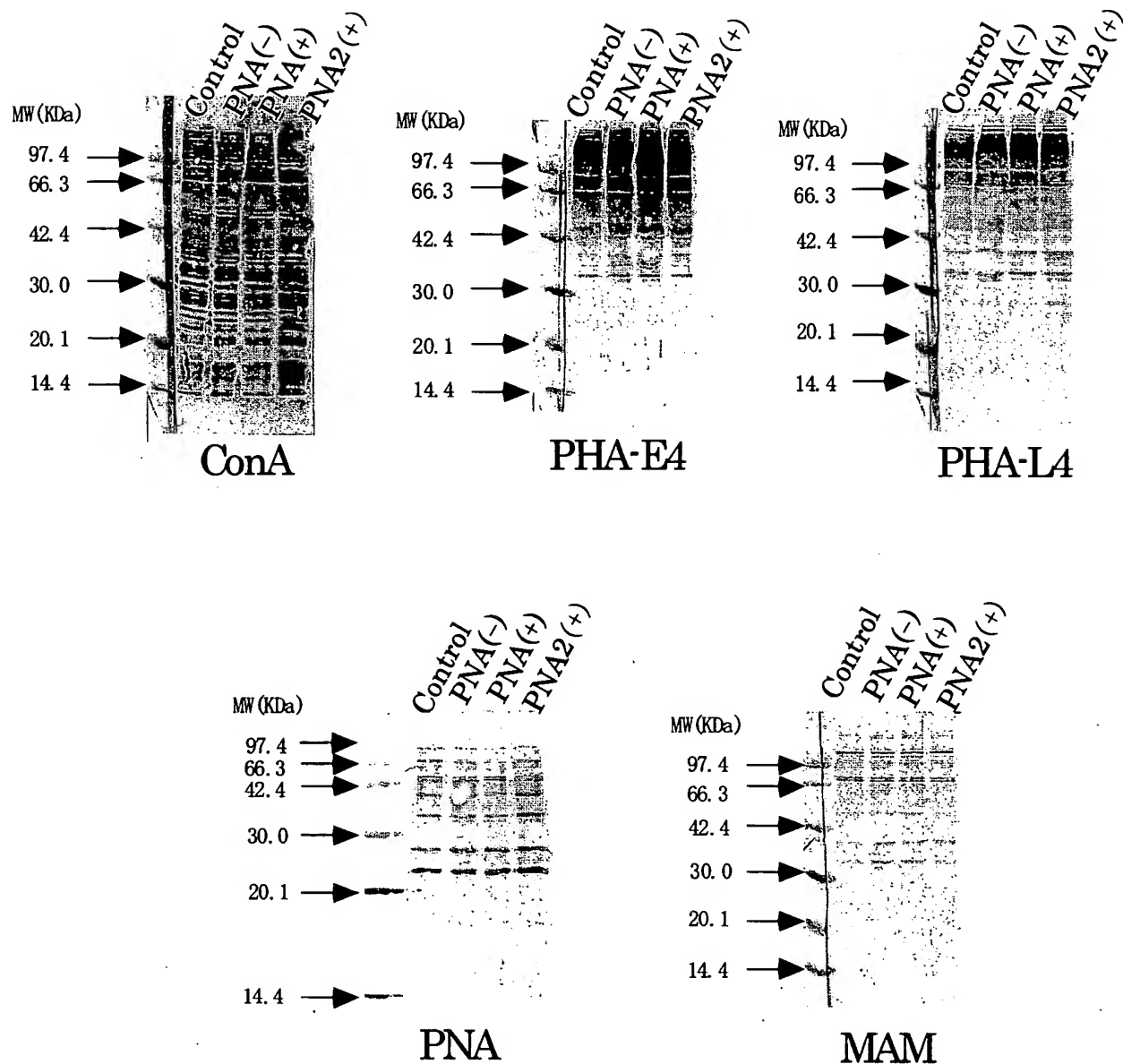
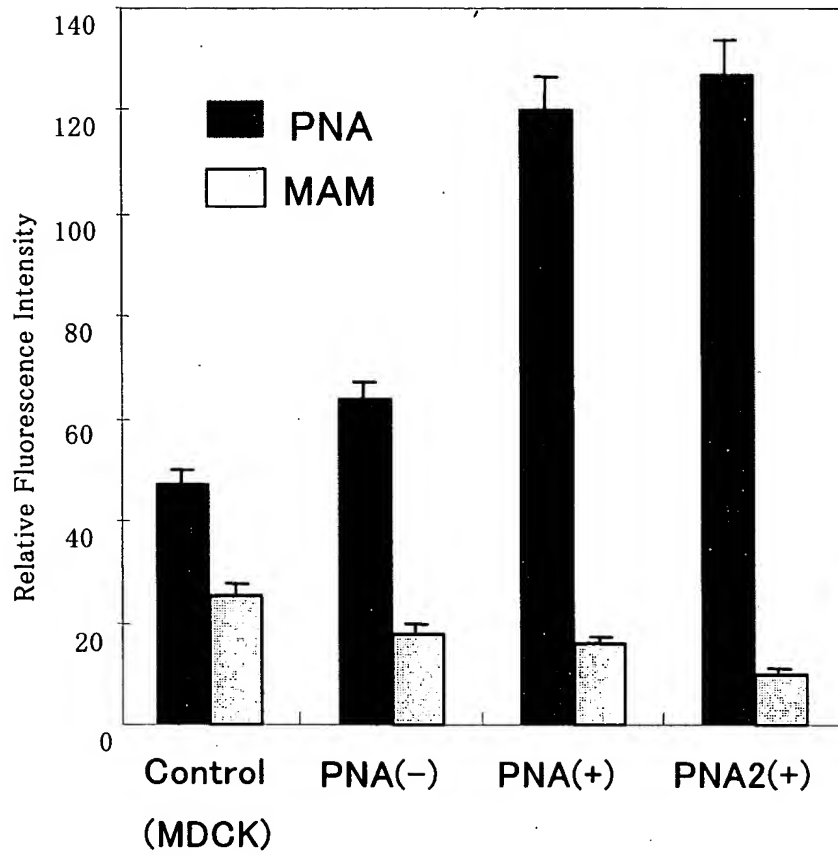


FIG 13

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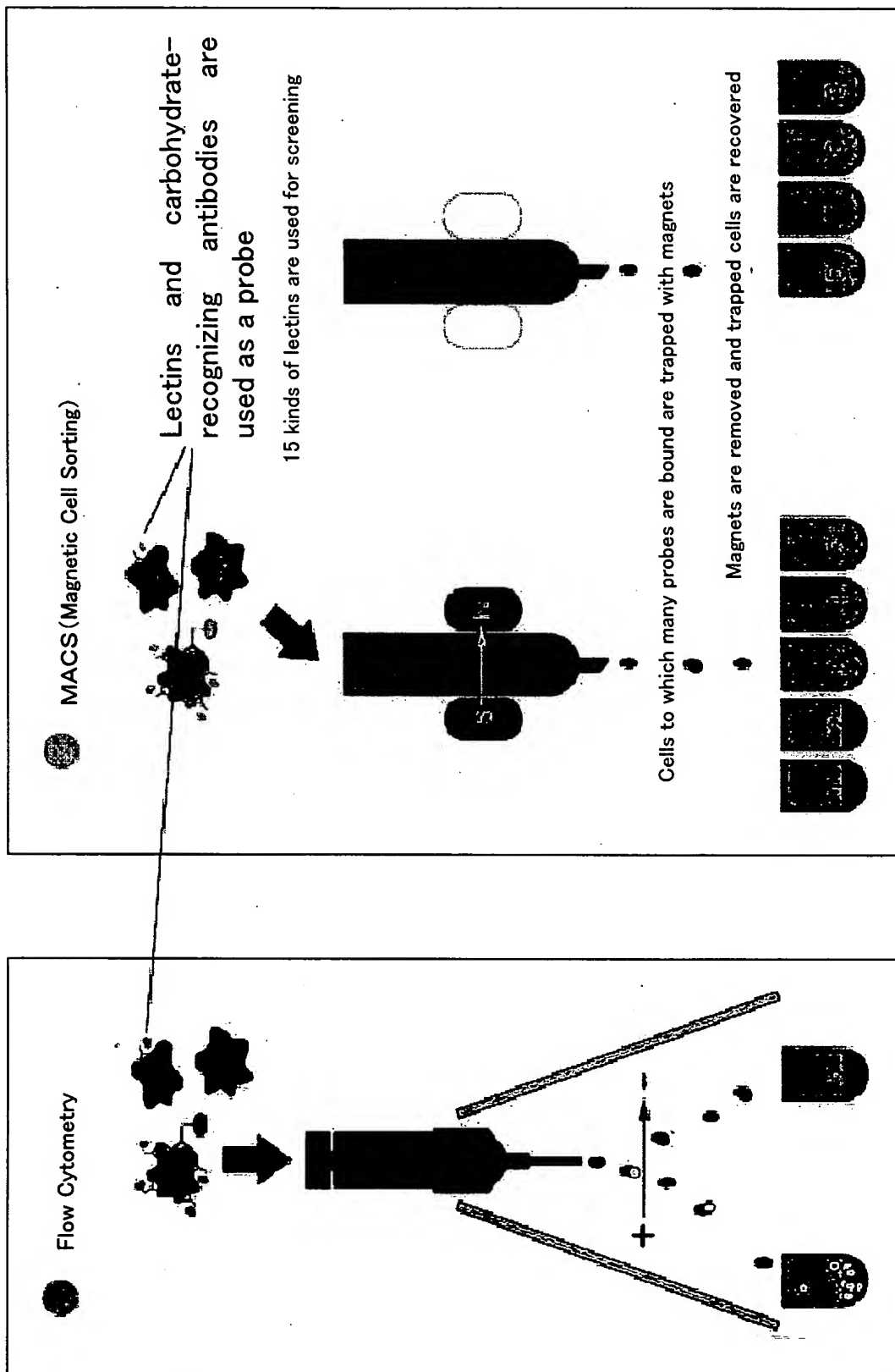


FIG 14

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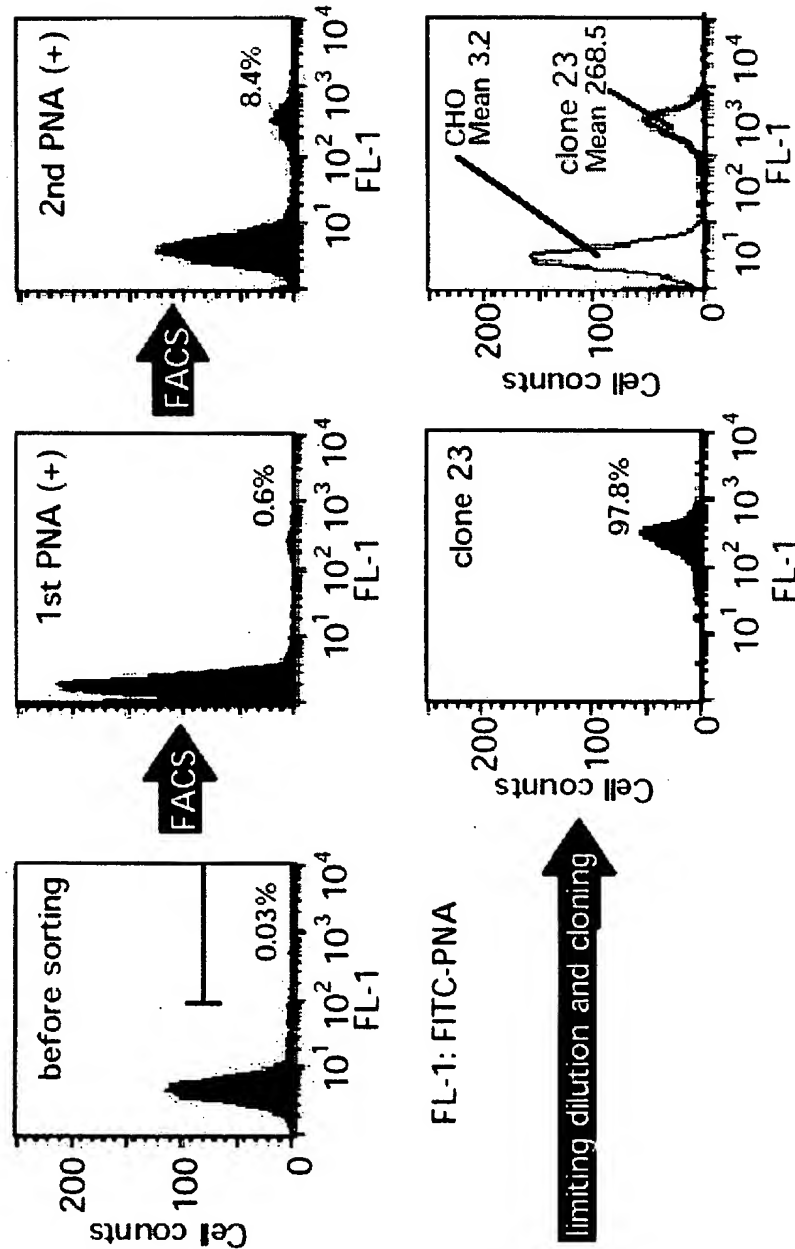


FIG 15

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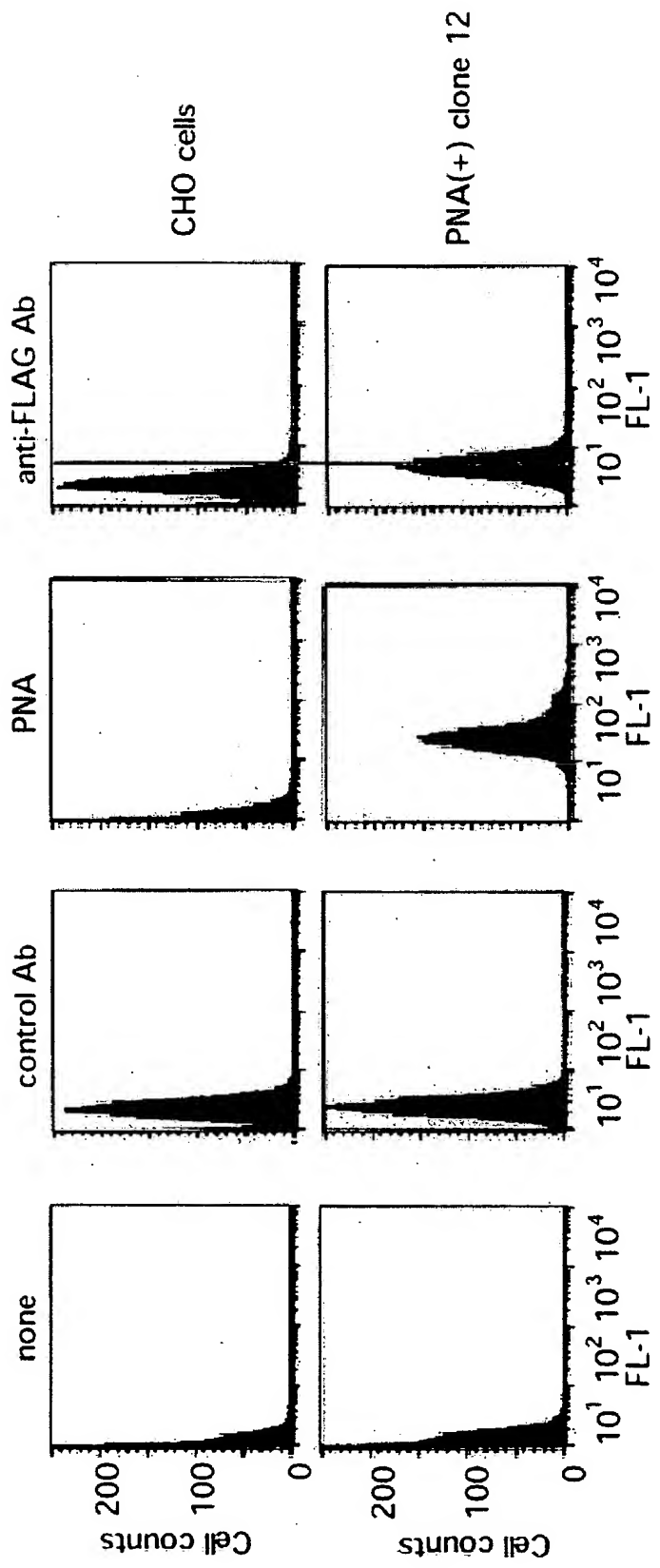


FIG 16

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